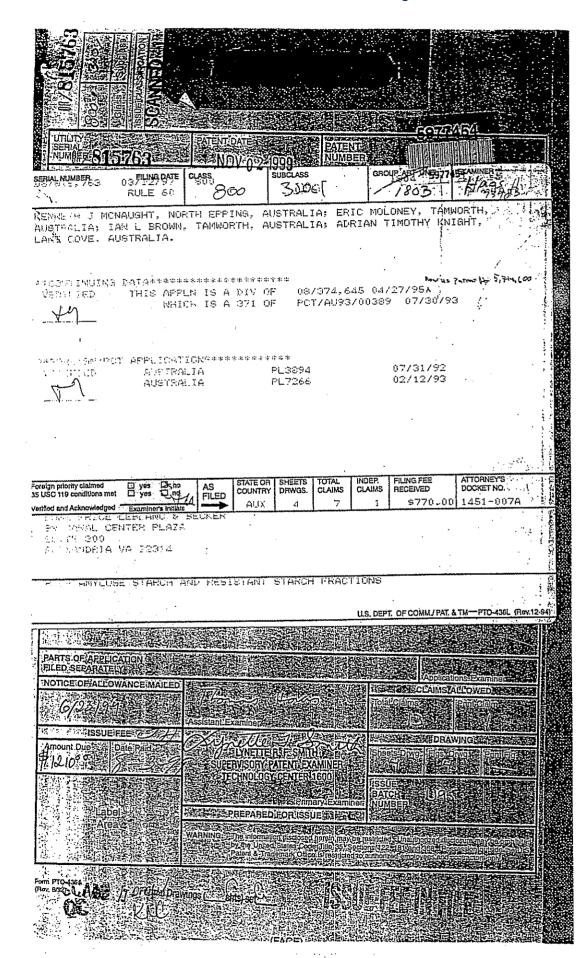
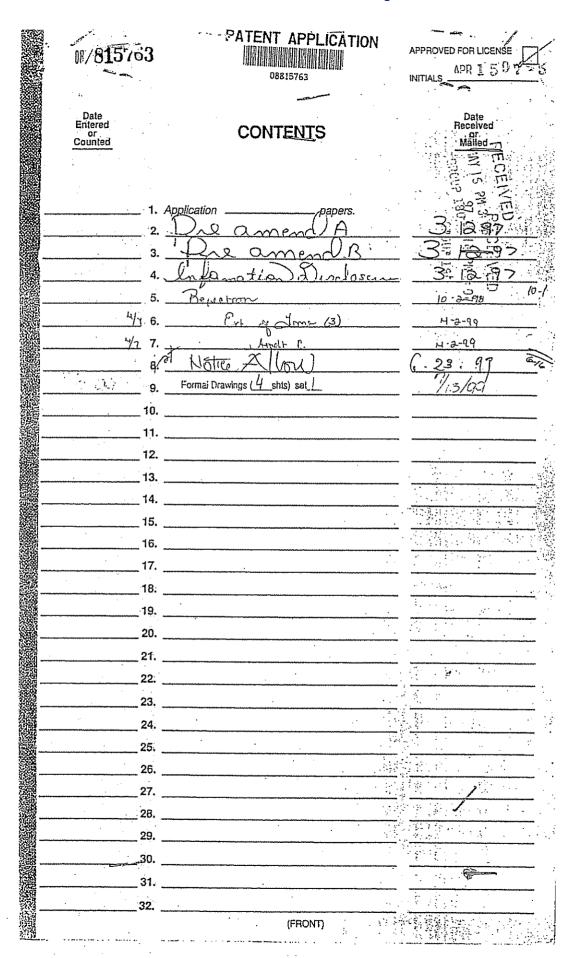
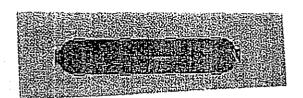
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PTO-1556 (5/87) WO 94/03049



- 1 -

### HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

#### Technical Field



This invention relates to high amylose content starch, in particular to a maize starch having an amylose content of more than 80% w/w. The invention further relates to single, double and multiple cross maize hybrids, particularly to a maize single cross F1 hybrid, capable of producing grain having such a high amylose content and to this grain.

The invention still further relates to fractions of high amylose starch that are enriched in dietary fibre and resistant starch content whilst claiming a high amylose content.

#### 15 Background Art

Most common starches contain approximately 25% amylose and 75% amylopectin. Amylose is a linear glucose polymer fraction, whilst amylopectin is a branched glucose polymer fraction.

In the prior art, it has been recognized that 20 currently available commercial starch having an elevated amylose content would impart certain desirable properties to various compositions including films, foods and industrial products. Accordingly, attempts have been made 25 in the prior art to produce high amylose content maize. This is exemplified in AU-A-45616/89 wherein a maize seed deposited as ATCC No. 40499 is disclosed as capable of yielding a starch having an amylose content of up to 72%.

Typically, however, a commercial starch having an 30 amylose content of 55-65% would be regarded in the art as having a high amylose content.

The present inventors whilst recognizing the utility of the commercially available so-called high amylose starches, have sought to produce a maize having a still 35 higher amylose content.

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#### Disclosure of Invention

In the course of a breeding program, a single cross F1 hybrid maize seed was produced, which carried the ae amylose extender gene. This seed was found to be capable 5 of producing grain, in which the amylose content of the starch derived therefrom was in excess of 80%.

Accordingly, in a first aspect, this invention consists in a hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

In a second aspect, this invention further consists in a maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.

In a third aspect, this invention still further consists in compositions including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof. -20

In a fourth aspect, this invention still further consists in a process for the formation of a composition comprising including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof, in said composition.

In a fifth aspect, the present invention still further consists in a hybrid maize seed resulting from a 30 cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than 80%.

PCT/AU93/00389

- 3 -

Starch granules from any botanical source are a heterogeneous mixture varying in physiological age and this affects their physical size, structure and properties. If the starch granules are physically separated according to their granule size, it has been noted by a number of authors that the properties of each size fraction are somewhat different. For example, Cluskey et al in Starke, 32, 105-109(1980) reported on the fractionation of dent corn and amylomaize starch granules. They found that an inverse relationship existed between granule size and iodine binding capacity in the amylomaizes. Thus, the percent apparent amylose found in the fractions of amylose V starch amounted to 40% for the largest size particles and 52% for the smallest particles.

The correlation between amylose content and size fraction has been observed by the present inventors in relation to high amylose starches of the type mentioned above and in co-pending patent application PL6537.

In this latter mentioned patent application, PL6537,

20 it was disclosed that high amylose starches have a high
dietary fibre or resistant starch content. More
specifically, it was found that there was a correlation
between amylose content and dietary fibre/resistant starch
such that increasing levels of amylose above 55% were

25 associated with increasing levels of dietary
fibre/resistant starch.

Patent application PL6537 further disclosed the useful nature of such starches in the preparation of food compositions having an enhanced dietary or resistant starch content.

Based on the observations of

- (1) an association of dietary fibre and resistant starch with increasing levels of amylose; and
- (2) increasing amylose content with decreasing starch 35 granule size,

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it was to be expected that decreasing starch granule size fractions of high amylose starch would be associated with enhanced levels of dietary fibre and resistant starch.

Surprisingly, this was found to be incorrect. In 5 fact it was found that there is an optimum starch granule size fraction which is intermediate in size and not necessarily associated with the highest amylose content fraction.

Accordingly in a sixth aspect, the present invention 10 still further consists in a starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which 15 is greater than said high amylose starch.

In a seventh aspect, the present invention still further consists in a food composition having an enhanced dietary fibre and/or resistant starch content, including a starch fraction of enhanced dietary fibre and/or resistant 20 starch content derived from a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

25 For the purpose of the description of this invention, "high amylose" means an amylose content (dsb) of 50% or more, preferably 70% or more, most preferably 80% or more. Particularly preferred amylose contents are 85% or more and 90% or more.

For the purposes of the description of the invention, the method by which amylose was determined is set out below.

HETHOD:

Apparent Amylose (Blue Value)

High Amylose Maize Starch

#### APPARATUS:

#### Defatting

Soxhlet extraction apparatus

Steam bath

Whatman thimbles, 25 x 80mm

Drying Oven 105°C

Desiccator

Amylose Determination

Stoppered 50ml test tubes

Vortex mixer

Boiling water bath

Spectrophotometer (605mm, slit width 0.2mm)

#### REAGENTS:

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#### Defatting

Methanol (AR Grade)

Amylose Determination

Dimethylsulfoxide (HPLC Grade)

Iodine/Potassium iodide solution

3.0g iodine and 30g potassium iodide made up to 1000mls with 0.1N sodium hydroxide.

Methanol (AR Grade)

Amylose (Sigma Cat. No AO512)

Dried for 2 hours at 105°C prior to use.

#### PROCEDURE:

#### Defatting

- (1) Weigh 5 grams of starch into the thimble.
- (2) Place the thimble in the Soxhlet apparatus.
- (3) Extract the sample with methanol (200mls) for 20 hours
- (4) Recover the thimble and dry in an oven at 105°C for 12 hours.

Amylose Determination

NAST BED

			(1)	Accurately weigh starch (100.0 to 105.0mg) into
				the text tube.
		٠	(2)	Add methanol (Iml) and vortex mix.
				Add DMSO (15mls), invert the test tube, and
		5		vortex mix.
		•	(4)	Place the test tubes in a vigorously boiling
			<i>;</i> • • • • • • • • • • • • • • • • • • •	water bath for 60 minutes.
			(5)	Invert and vortex mix each test tube at 15 minut
				intervals during this period.
		10	(6)	Add distilled water (15mls), invert and vortex
	•			mix. Place the test tube in the boiling water
				bath for a further 30 minutes.
とられるない。			(7)	Quantitatively transfer the contents of the test
Ö.				tube to a 100ml volumetric flask (use a funnel
- <u>-</u>		15		in the flask). Make the solution to volume with
⊎: \}				distilled water.
(T)			(8)	Transfer an aliquot (3mls) of this solution to a
li) '			•	100ml volumetric flask and add 90mls of
] ,j				distilled water.
ep Tj		20	(9)	Add Iodine/Potassium Iodide solution (lml) to th
				diluted solution and immediately shake and mix
V D			•	thoroughly. Make to volume with distilled water
<b>√</b> i			(10)	Measure the absorbance of this solution at 605 m
				compared to a blank consisting of Iodine/
		25		Potassium Todide solution (lml) diluted to 100ml
				with distilled water in a volumetric flask.
			CALCULATIO	<u> </u>
	٠.		- 12 <b>-</b> - 1	

30 Absorbance x 13 % Amylose dsb wt. sample dsb

\* dsb = dry solids basis.

The method by which starch was separated from the 35 maize grain was as follows:-

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- Prepare 200g meal by grinding through the 2mm then 1. the lmm screen of he Retsch Mill.
- Wet thoroughly, stirring by hand, with 600ml 0.1N NaOH. 2.
- Add 2,200ml 0.1N NaOH and blend 5 minutes at 2/3 3.
- 5 speed with the Ultra Turrax.
  - 4 Sieve over 44u screen.
  - Return sieve overs with 1L water and blend for another 3 minutes, if necessary.
  - 6. Sieve over 44u screen.
- 10 7. Centrifuge filtrate at 3000 rpm for 15 minutes. Decant. Wipe out the neck of the bottle with a tissue to remove fat.
- 8. Reslurry starch (centrifugate) with 200ml water, i.e. 50ml in each of 4 tubes. Centrifuge.
- Remove starch from centrifuge tubes with about 250ml 15 9.
  - 10. Adjust pH of starch slurry to 6.0-6.5 with 0.5N HCl. Filter again over 44u screen, if necessary.
  - 11. Buchner filter and air dry.
- 20 Modes for Carrying out the Invention

In order to better understand the nature of this invention, a number of examples will be described.

#### Brief Description of Drawings

Fig. 1 is a gel permeation chromatography molecular

25 weight profile of a number of maize starches;

Fig. 2 is a viscograph of a number of maize starches in water;

Fig. 3 is a viscograph of a number of maize starches in base: and

30 Fig. 4 is a graph of total dietary fibre versus average starch granule size.

#### Maize Seed

A range of parental lines of maize seeds were obtained from High Yield Seed Co, Tamworth, Australia.

35 Non-limiting examples of these parenting lines included

Form PCT/IDALLIVO

G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144.

Hybrids were produced by crossing inbred lines

5 carrying the <u>ae</u> amylose extender genes. These inbred
lines were selected for combining ability and identified
as specific female and male parents to produce the
hybrids. Conventional breeding methods and techniques
were used in developing inbred lines with repetitive

10 amylose assays to ensure the transfer of recessively
inherited <u>ae</u> gene.

One particular cross between male G116 and female G121 resulted in a F1 hybrid, referred to as Code 008 and deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD20853, U.S.A., under the designation 75182 on 15 January 1992. This hybrid yielded grain the starch of which was found to have an amylose content in excess of 80%.

Based on the disclosure of this invention, the person skilled in the art would expect that hybrids resulting from further crosses of the parental lines mentioned above will yield starch having an amylose content in excess of 80%.

In fact experimental hybrids have yielded starches

25 obtained from crosses between the above mentioned parent
lines having high amylose contents. Set out below is a
summary of the relevant crosses with amylose content in %
bracketed.

	<u>FEMA</u>	LE	MALE .	HYBRID
	1.	G117 (81.6)	G116 (82.2)	G117 x G116 (83.3)
	2.	G116 (82.2)	G122 (89.6)	G116 x G122 (80.5)
	3.	G118 (94.3)	G122 (89.6)	G118 x G122 (85.9)
5	4.	G120 (94.6)	G122 (89.6)	$G120 \times G122 (80.4)$
	5.	G122 (89.6)	G120 (94.6)	G122 x G120 (81.9)
	6.	G122 (89.6)	G140 (92.2)	G122 x G140 (85.4)
:	7.	G128 (71.5)	G129 (61.8)	G128 x G129 (82.8)
	8.	G140 (93.2)	G121 (94.7)	G140 x.G121 (93.0)
10	9.	G140 (92.2)	G144 (60.4)	G140 x G144 (85.3)
	* 10.	G139W (71.9)	G136W (93.4)	G139W x G136W (95.7)
	11.	G121 (94.7)	G126 (82.2)	G121 x G116 (85.0)

= White seed.

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Experiments conducted using Code 008 seed have shown that the climatic and agronomic conditions under which the maize is grown will have a significant effect on the amylose content. Specifically, it has been found that seed 20 cultivated under irrigation near Tamworth, Australia (latitude 31.10S) in an early crop and a late crop yielded starch having an amylose content respectively of 85.0% and 90.1%. Similarly, a crop cultivated at Finley, Australia (latitude 35.6°S) yielded starch having an amylose content 25 of 94.8%. By contrast, the same seed when sultivated under irrigation at Giru, Australia (latitude 19.5°) yielded a starch having an amylose content of 78.6%.

Accordingly, a preferred embodiment of this invention comprises a maize seed deposited with the ATCC and 30 designated 75182.

A further preferred embodiment of this invention comprises a maize starch having an amylose content of 85.0% or more, most preferably 90.1% or more.

To further characterize the maize starch derived from Code 008 grain, molecular weight profiling by gel

permeation chromatography has been performed. The method by which this was done is set out below whilst the results are shown in the accompanying Figure 1. For comparative purposes, two commercially available maize starches, HA 5 Class V and HA Class VII are shown.

METHOD:

Gel Permeation Chromatography of Starch

Starch SCOPE:

#### APPARATUS:

#### Sample Preparation

Pump

Injector

Screw capped test tubes (50ml) 10 Boiling water bath Microcentrifuge (Eppendorf 5415) Desiccator

#### HPLC

Alltech GPC High MW Polar 5U 15 Column (Cat. No. 100586)

> Waters 410 Refractive Index Detector Detector (X 128 35°C)

Waters 600 E Waters 712 WISP

Column Heater (Set at 25°C) Maxima 825 (V 3.3) Software

#### REAGENTS:

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Dimethyl sulfoxide (Chrom AR HPLC Grade -Mallinckrodt) Dimethyl formamide (Chrom AR HPLC Grade -Mallinckrodt)

Pullulan Molecular Weight Standards - Showa Denko (ex Edward Instruments)

HPLC Mobile Phase - DMSO:DMF (20:80)

#### SAMPLE PREPARATION:

#### Standards

(1) The pullulan molecular weight standards need to be weighed into the screw capped test tubes in the following manner:

Tube	1		5.0mg	each	of	P800,	P100,	P10	and
gluco	)S E	9							

Tube 2 - 7.0mg each of P400, P50 and P5

Tube 3 - 7.0mg each of P200, P20 and maltotriose.

- (2) Add DMSO (4mls) to each tube and tightly seal it.
- heat the tubes in the boiling water bath for 5 minutes to dissolve the pullulan.
- Remove and cool the test tube to room temperature.
- (5) Add DMF (16mls) and mix well.
  - (6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
  - (7) Remove the top 1ml of solution from each centrifuge tube and place in a WISP vial.

#### 15 Samples

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- (1) Accurately weight the sample (50.0mg) into a screw capped test tube.
- (2) Add DMSO (10mls).
- (3) Heat in a boiling water bath for 60 minutes.
- 20 (4) Remove and cool the test tube to room temperature.
  - (5) Add DMF (40mls) and mix well.
  - (6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
- 25 (7) Remove the top lml of solution from each centrifuge tube and place in a WISP vial.

#### HPLC Preparation

- (1) Prior to fitting the column, pump water (100mls) through the HPLC.
- (2) Prepare the mobile phase and pump 50mls through the HPLC. Ensure that the WISP is purged during this stage.
  - (3) Adjust the flow rate of 0.2ml/minute and connect the column.
  - (4) Allow the column to equilibrate overnight.

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- (5) Prior to the injection of samples, purge the WISP and then gradually increase the flow rate to 1.5mls/minute.
- Set the column heater to 25°C. (6)
- Inject the standards and samples 100µl injection volume.
  - (8) After samples have been analysed turn the column heater off and reduce the flow rate of 0.2mls/minute.
- 10 (9) Disconnect the column.
  - (10) Wash the system with water overnight at 0.5mls/minute.
  - (11) Wash the system with methanol (200mls).

Viscographs have also been prepared comparing maize 15 starch from Code 008 (designated Gelose 80) with Gelose 50 and Gelose 70. Figure 2 shows the viscosity profile under alkaline conditions whilst Figure 3 shows the viscosity profile in water.

#### Maize Starch

The maize starch of the first aspect of this 20 invention having an amylose content of more than 80% may be used in a variety of compositions known in the art. The usefulness of the starch is believed to be a result of the higher content of more linear molecules. This seems 25 to impart physical properties which tend towards those of conventionally used synthetic plastics materials. Consequently, films formed from the starch of the invention have higher tensile strengths and are good oxygen barriers. The starch is also easier to process on 30 existing synthetic plastics materials equipment such as blow moulding and injection moulding machines.

Furthermore, this starch may be physically modified or chemically modified to produce a variety of derivatives well known in the art. These starches may also be used in 35 a variety of compositions.

Finally, this starch may also be used in processes and compositions requiring the starch to be destructurized within the meaning of that term defined in EP0118240.

Some non-limiting examples of compositions in which 5 the maize starch of this invention in all of its forms, could be used include:

- Corrugating adhesives.
- 2. Sausage skins.
- Confectionery. 3.
- 10 Other food compositions where the enhanced gel strength of the starch would be advantageous.
  - Films, either alone or laminated with polymers such as ethylenevinylalcohol to achieve both gas and water barrier properties.
- Biodegradable and controlled release matrices and 15 6. methods for forming and using these matrices as disclosed in PCT/AU90/00422, the contents of which is incorporated herein by way of reference.
- 7 Shaped articles, processes for forming shaped 20 Particles and methods for using shaped articles as disclosed in PCT/AU90/00237, the contents of which is incorporated herein by way of reference.
  - Coextrusions with synthetic polymers.
- 9. Intermediate products such as pellets and rods, 25 formed for example by extrusion, and including combinations of starch with one or more natural or synthetic polymers, plasticizers, colourants and other additives.
- 10. Other blends of starch with natural or synthetic 30 polymers to obtain enhanced structural properties.

#### Starch Fractions

The starches of the sixth and seventh aspects of this invention may originate from a number of sources including cereals such as maize, barley, wheat and legumes, providing 35 that the starch content of such sources is high in amylose.

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To fractionate the starch granules, there are a number of methods known in the art including dry powder sieving, hydrocyclone classification, air classification and differential sedimentation. A person skilled in the 5 art would be readily able to choose an appropriate method depending on the source material and other relevant factors.

Although the size fraction of enhanced dietary fibre and/or resistant starch may vary, the example that follows 10 describes the work that was done by the present inventors in relation to a maize starch sample. Based on this disclosure, a person skilled in the art could readily repeat this work using other starch sources to identify an appropriate fraction.

Once the starch has been appropriately fractionated, the fractions having enhanced dietary fibre and/or resistant starch content may be processed to obtain starch having further increased dietary fibre and/or resistant starch content using entirely conventional methods well

20 known in the art. An example of the fractionation will now be described.

#### Fractionation of Maize Starch by Granule Size

A high amylose maize starch - High Amylose 80(10/91) was fractionated into seven subsamples based on granule 25 size using the aqueous differential sedimentation procedure described by Cluskey et al (1980). This method was chosen since it minimised damage to the starch, did not introduce any residues and it was indicated that exposure of the starch granules to distilled water for long periods of time did not affect their integrity. Each subsample was weighed, measured for average granule size and the apparent amylose content, total dietary fibre and resistant starch determined. Each starch sample (60 grams) was separated into the seven fractions which 35 were freeze-dried and weighed on a Mettler PE 3600 top pan balance. A scanning electron microscope was used to visually check the uniformity of the size distribution of the granules in each fraction.

Each fractionated starch sample was analysed for 5 granule size according to the method described below. Apparent amylose content was determined using the method described above. Dietary fibre and resistant starch (McCleary et al) were determined using the methods disclosed in co-pending application PL6537.

Granule size was determined using a Malvern Master Sizer which utilises a He-Ne laser (632.8nm) with a maximum output of 5mW CW. In this method a starch slurry was made using approximately 15mL of distilled water in a 50mL beaker. The slurry was sonicated for 4 minutes. The 15 slurry was then introduced into the stirred cell and the obscuration value adjusted using distilled water to 0.20. The slurry was allowed to stir for a further 2 minutes before readings were taken. Four readings were taken for each sample in order to check the stability of the 20 readings being obtained.

#### Results

In Table 1 set out below, there is shown the results (the average of two separate fractionations, together with the range of analytical results) obtained for each of 25 seven particle size fractions. These results are graphically presented in Fig.4, from which it is particularly evident that the level of resistant starch and dietary fibre is significantly increased between the second and fifth fractions, ie, 10.2-7.6 microns. Thus, 30 if those starch fractions were to be segregated from the original starch sample, only 46.9% of the solids would need to be removed to produce a fraction in which the resistant starch was increased by 36% and dietary fibre by 24%.

Although the starch fractions of the invention are 35

	•				
	Amount in Fraction	Average Granule Size	Apparent Anylose Content	Total Dietary Fibre	Resistant Starch
	(I) dsb	(microns)	(%) dsb	(I) dsb	(I) dsb
High Amylose 80 - 10/91	100.00	10.0	85	33.4	18.1
Fraction 1	35.6 ± 1.1	$12.3 \pm 0.5$	80 ± 0	31.4 ± 1.5	17.7
Fraction 2	15.0 ± 2.6	10.2 ± 0.1	83 ± 1	38.3 ± 2.0	16.4
Fraction 3	13.0 ± 1.1	9.1 ± 0.2	85.5 ± 0.5	41.3 ± 0.3	22.8
Fraction 4	14.9 ± 1.0	8.3 ± 0.1	85.5 ± 0.5	39.4 ± 4.1	24.6
Fraction 5	10.2 ± 1.6	7.6 ± 0.1	88.5 ± 0.5	37.2 ± 1.3	18.9
Fraction 6	7.0 ± 1.6	7.2 <u>+</u> 0.1	89.5 ± 0.5	31.3 ± 2.4	21.7
Fraction 7	4.3 ± 2.7	6.8 ± 0.2	89	28.1	10.1

high in dietary fibre and/or resistant starch, it should also be appreciated that another important property is that these fractions are "naturally" derived. This arises out of the fact that the fractions are prepared using a 5 physical means of separation. No chemical or other treatments are required in order to produce starch fractions having a high dietary fibre and/or resistant starch content. Such a property is of particular importance in food applications in that no regulatory approval would be required in order to incorporate such materials in food compositions.

The person skilled in the art will readily appreciate that the starch fractions of the invention having the enhanced dietary fibre and/or resistant starch content may 15 be used in a variety of food compositions. Such uses are disclosed, for example, in co-pending application No PL6537.

Whilst it is not as yet known why the fractions of the invention have enhanced dietary fibre and/or resistant 20 starch content, it will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described without departing from the spirit or scope of the invention as broadly described. Accordingly, the Example based on a 25 sample of high amylose maize starch is to be considered in all respects as illustrative and not restrictive.

The person skilled in the art will readily appreciate that the maize starch of the invention both in its native form, and the other forms mentioned above will have many 30 applications additional to those mentioned.

It will also be appreciated by those skilled in the art that numerous variations and modifications may be made to this invention without departing from the spirit or scope thereof as broadly described.

Document 44-4

- A hybrid maize seed capable of producing a starch having an amylose content of more than 80%.
- A hybrid maize seed as in claim 1 obtained from a 5 cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed Yielding a starch having an amylose content of more than 10 80%.
  - A hybrid maize seed as in claim 2 selected from the 3. group consisting of the following crosses: G117 x G116, G116 x G122, G118 x G122, G170 x G122, G112 x G120, G122 x G140, G128 x G129, 9140 x G121, G140 x G144,
  - A hybrid maize seed as in any one of claims 1 to 3 G139W x G136W and G121 x 6116. wherein the seed yields a starch having an amylose content of 85.0% or more.
- A hybrid maize seed as in claim 4 wherein the seed 20 yields a starch having an amylose content of 90.1% or more.
  - A hybrid maize seed as in claim 5 wherein the seed yields a starch having an amylose content of 94.8% or more. A hyprid maize seed as in claim 3 deposited as ATCC
- 75182. A maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destitucturized and non-destructurized forms
  - thereof. A maize starch as in claim 8 having an amylose
- 30 content of 85.0% or more. 10. A maize starth as in claim 9 having an amylose content of 90.1% br more,
  - A maize starth as in claim 10 having an amylose content of 94.8% or more.
- 12. A composition including a maize starch selected from

the group consisting of maize starch having an amylose content of more, than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof.

- 5 13. A composition as in claim 12 wherein the maize starch has an amylose content of 85.0% or more.
  - 14. A composition as in claim 13 wherein the maize starch has an amylose content of 90.1% or more.
- 15. A composition as in claim 14 wherein the maize starch 10 has an amylose content of 94.8% or more.
  - 16. A starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch, the amylose content of which is 50% or more, which has been fractionated according to granule size to yield a
- 15 fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.
- 17. A starch fraction as in claim 16 wherein the high amylose starch is selected from the group consisting of 20 maize, barley, wheat and legumes.
  - 18. A starch fraction as in claim 16 or claim 17 wherein the amylose dontent of the high amylose starch is 70% or more, preferably 80% or more.
- 19. A starch praction as in claim 18 wherein the amylose 25 content of the high amylose starch is 85% or more, preferably 90% or more.
  - 20. A starch fraction as in any one of claims 16 to 19 wherein the fractionation is by dry powder sieving, hydrocyclone classification, air classification or
- 30 differential sedimentation. 21. A starch fraction as in any one of claims 16 to 20 wherein the dietary fibre content of the fraction is increased by about 24% or more and the resistant starch content of the fraction is increased by about 36% or more 35 over the high amylose starches.

- A starch fraction as in any one of claims 16 to 21 wherein the average granule size of the fraction is from about 10.2 to 7.6 microns.
- A food composition including a starch fraction as claimed in any one of claims 16 to 22.

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## DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare than

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled, the specification of which HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

[] is attached hereto. [I] was filed on as Application Serial No. and was amended on (if applicable)

PCT/AIJ93/00389 filed 30 July 1993

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Priority Claimed Prior Foreign Application(s): Country Day/Month/Year filed Australia 31 July 1992 Australia 12 February 1993 Number PL 3894 Yes Ō (1) PL 7266 j.

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Status: Patented, Pending, Abandoned Application Serial No. Filing Date

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s): Allan M. Lowe, Reg. No. 19,641; Robert L. Price, Reg. No. 22,685; Robert E. LeBlanc, Reg. No. 17,219; Stephen A. Becker, Reg. No. 26,527; Henry Shur, Reg. No. 17,414; Israel Gopstein, Reg. No. 27,333; Benjamin J. Hauptman, Reg. No. 29,310; Donald C. Casey, Reg. No. 24,022; Kenneth E. Krosin, Reg. No. 25,735; Chitaranjan N. Nirmel, Reg. No. 30,408; Holly D. Kozlowski, Reg. No. 30,468; Gene Z. Rubinson, Reg. No. 33,351; Frank P. Presta; Reg. No. 19,628; Michael S. Gzybowski, Reg. No. 32,816; Robert G. Lev, Reg. No. 30,280; Keith E. George, Reg. No. 34,111; Arthur P. Demers, Reg. No. 32,660; Edward J. Wise, Reg. No. 34,523; Christopher W. Brody, Reg. No. 33,613; Demetra J. Mills, Reg. No. 24,500; Daniel Y.I. Kim Beg. No. 35,508; Robert P. Bell 34,506; Daniel Y.J. Kim, Reg. No. 36,186; Alexander Yampolsky, Reg. No. 36,324; Sharon E. Finkel, Reg. No. 35,798; Robert P. Bell, Reg. No. 34,546; and Alfred A. Stadnicki, Reg. No. 30,225. all of

> LOWE, PRICE, LEBLANC & BECKER 99 Canal Center Plaza, Suite 300 Alexandria, Virginia 22314

with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

	***************************************
Full name of sole or first inventor. Kenneth LMcNAUGHT Inventor's Signature Km manfut	Date + 3 ganway 19
Residence: 18 Marcella Street, North Eppino	
Post Office Address: As above	2 1 2 2 2
	nage 1 of 2

page 1 of 2

	page 2 of 2
	PCT/AU93/00389 filed 30 July 1993
-	HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS
150	Full Name of Second Inventor: Eric HALONEY Edm. 11/4/95.  Inventor's Signature: E.R.Moleney Date: 22/12/94.
L .	Inventor's Signature: x E. R. Molone, Date: 22/12/94.
	Residence: 169 Brisbane Street, Tamworth, NSW 2340, Australia /-/(/
	Citizenship: Australian
	Post Office Address: As above
	Full Name of Third Inventor: Ian L BROWN
m	Inventor's Signature: Date:
	Residence:
	Citizenship:
n J n	Post Office Address:
ή	
P	Full Name of Fourth Inventor: Adrian Timothy KNIGHT
<del>7</del> 1}	Inventor's Signature: Date:
) ]	Residence:
Ţ	Citizenship:
	Post Office Address:

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	page 2 of 2
	PCT/AU93/00389 filed 30 July 1993
	HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS
*.	Full Name of Second Inventor: Eric MALONEY
	Inventor's Signature: Date:
	Residence:
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	Post Office Address:
3-07	Full Name of Third Inventor: Ian L BROWN  Inventor's Signature: A Date: > 15/12/94
I I	Residence: 2 Melissa Avenue, Tamworth, NSW 2340, Australia
ŭ.	Citizenship: <u>"Australian</u>
N J	Post Office Address: As above
	Full Name of Fourth Inventor: Adrian Timothy KNIGHT
	Inventor's Signature: Date:
	Residence:
) J	Citizenship:
	Post Office Address:

1.1.023

	page 2 of 2				
	PCT/AU93/00389 filed 30 July 1993				
	HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS				
	Full Name of Second Inventor: Eric MALONEY	•			
•	Inventor's Signature:	Date:			
-	Residence:				
	Citizenship:				
	Post Office Address:				
	Full Name of Third Inventor: Ian L BROWN				
max.	Inventor's Signature:	Date:			
	Residence:				
	Citizenship:				
1	Post Office Address:				
n ₫					
1400	Full Name of Fourth Inventor: Adrian Timothy KNI				
j / 0 ±	Inventor's Signature:x AThungh				
	Residence: 18 Nundah Street, Lane Cove, NSW 2066	, Australia ////			
	Citizenship: Australian				
	Post Office Address: <u>As above</u>				

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PCT/AU93/00389

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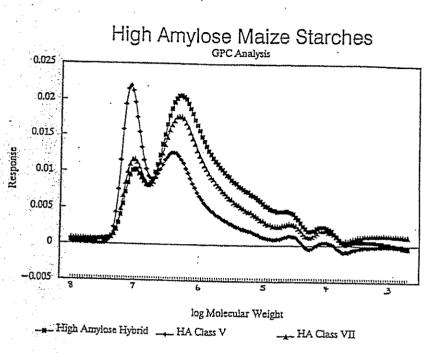
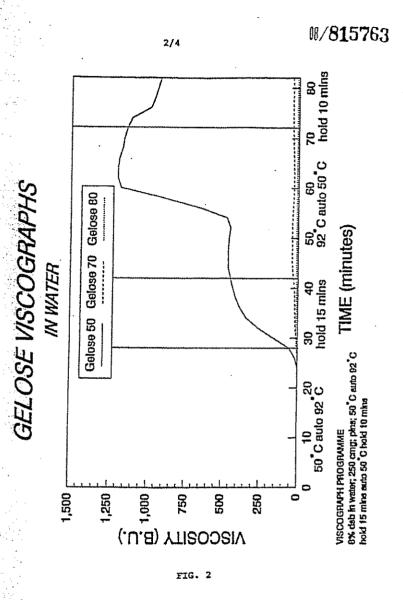


FIG. 1





SUBSTITUTE SHEET

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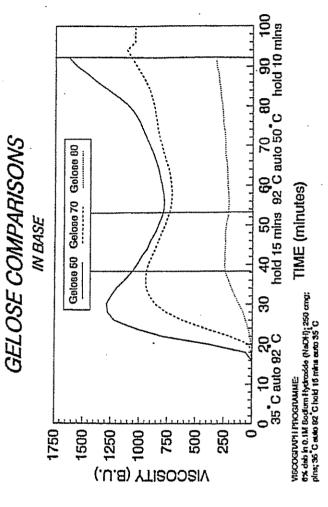
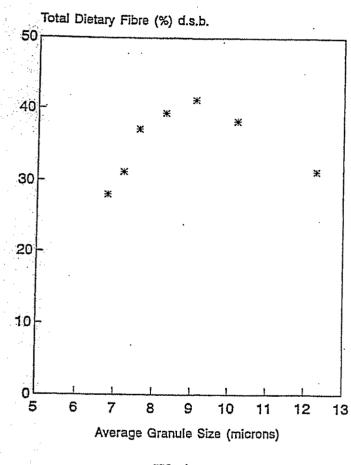


FIG. 3

4/4

Total Dietary Fibre Content of High Amylose Maize Starch Fractions High Amylose 80 (10/91)



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FIG. 4



# REQUEST FORM FOR DIVISIONAL APPLICATION UNDER 37 CFR 1.60

08/**81**5**7**63

pplication:

Art Unit: Examiner:

Assistant Commissioner for Patents Washington, DC 20231.

1804

Sir:

į

This is a Request for filing a Divisional application under 37 CFR 1.60 of pending prior application Serial No. 08/374,645, filed on April 27, 1995, entitled HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS .

by the following named inventor(s):

Ī	inventor	RESIDENCE	CITIZENSHIP	POST OFFICE ADDRESS
	Kenneth J. McNAUGHT et al.	North Epping, NSW 2121 AUSTRALIA	Australia <sub>.</sub>	18 Marcella Street North Epping NSW 2121 AUSTRALIA
ji Ul	Eric MOLONEY	Tamworth, NSW 2340 AUSTRALIA	Australia	169 Brisbone Street Tamworth, NSW 2340 AUSTRALIA
្រ ព្រ	Ian L. BROWN	Tamworth, NSW 2340 AUSTRALIA	Australia	2 Melissa Avenue Tamworth, NSW 2340 AUSTRALIA
	Adrian Timothy KNIGHT	Lane Cove NSW 2066 AUSTRALIA	Australia	18 Nundah Street Lane Cove, NSW 2066 AUSTRALIA

- I hereby verify, to the best of my knowledge, that the enclosed copy of this prior application is a true copy of the above-identified prior application, including the oath or declaration as originally filed. i. [X]
- 2. [X] Preliminary Amendment is enclosed.
- An Information Disclosure Statement and PTO1449 Form are submitted herewith.
- 3. [X] Cancel claims 8-23.

Page 37 of 73

4. The filing fee is calculated on the basis of the claims existing in the prior application as amended at 2 and 3 above;

	NO. OF CLAIMS		EXTRA CLAIMS	RATE	FEE
Total Claims	7	MINUS 20	0	x \$22 =	\$00.00
Independent Claims	1	MINUS 3	0	x \$78 =	0.00
		1	Basic A	pplication Fee	770.00
I	f multiple de	pendent claims	are presented	, add \$250.00	
			Total A	pplication Fee	770.00
			Subtract 1/2	if small entity	
		TOTAL A	APPLICATION	ON FEE DUE	\$770.00
AMOUNT TO BE	CHARGED	TO DEPOSE	ACCOUNT	NO. 12-2237	\$770.00

- Enclosed is a Verified Statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27. AAGHED EQASTERD 4a. [] A verified Statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27 was filed in prior 4b.[] application and such status is still proper and desired. The Commissioner is hereby authorized to charge fees under 37 CFR 1.16 and 1.17 which may be required, 5. [X] including any extension of time fees to maintain the pendency of the parent application Serial No. 08/374,645 or credit any overpayment to Deposit Account No. 12-2237. Amend the specification by inserting before the first line the sentence: 6. [X] -This application is a division of Application Serial No. 08/374,645 filed April 27, 1995, which is a filing under 35 USC 371 of PCT/AU93/00389, filed July 30, 1993.-
  - Priority of Application No. PL 3894, filed on July 31, 1992 and PL 7266, filed on February 12, 1993, in Australia 7. [X] is claimed under 35 USC 119. The certified priority document(s) were transmitted by WIPO and acknowledged as received in U.S. Serial No. 08/374,645.
  - The prior application is assigned of record to Goodman Fielder Limited. 8. [X]
  - The power of attorney in the prior application is to: 9. [X]

LOWE, PRICE, LEBLANC & BECKER

Also enclosed:

Four (4) pages of drawings.

A petition, fee and response has been filed to extend the term in the pending prior application until . 11.[]

Address all future communications to: (May only be completed by applicant, or attorney or agent of record)

LOWE PRICE LEBLANC & BECKER 99 Canal Center Plaza, Suite 300
Alexandria, Virginia, 22314

Respectfully submitted,

LOWE, PRICE, LEBLANC & BECKER

Robert L. Price

Registration No. 22,685

reared.

99 Canal Center Plaza, Suite 300 Alexandria, Virginia 22314 (703) 684-1111 RLP:brca Date: March 12, 1997

08/815763

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Kenneth J. McNAUGHT et al

Serial No.

Filed: March 12, 1997

Group Art Unit: 12-07

Examiner: NUTT car

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

# PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks Washington, D. C. 20231

Sir:

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Prior to examination on the merits, please amend the aboveidentified application as follows:

IN THE CLAIMS:

line 1, change "any one of claims 1 to 3" to --claim

#### REMARKS

The above-identified amendment to claim 4 is to merely delete the multiple dependency of the claim and thereby avoid the multiple dependency claim fee. Entry is respectfully submitted.

Respectfully submitted.

LOWE, PRICE, LEBLANC & BECKER

Robert L. Price

Registration No. 22,685

99 Canal Center Plaza, Suite 300 Alexandria, Virginia 22314 Phone: (703) 684-1111 Fax: (703) 684-1124 RLP:kmb

Date: March 12, 1997



#### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Kenneth J. McNAUGHT et al

Serial No. 81876

Filed: March 12, 1997

Group Art Unit: 1207 Examiner: Ny7724

For: HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

### INFORMATION DISCLOSURE STATEMENT

Honorable Commissioner of Patents and Trademarks Washington, D. C. 20231

Sir:

In accordance with the provisions of 37 C.F.R. 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached form PTO-1449. It is respectfully requested that the references be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is being filed within three months of the U.S. filing date OR before the mailing date of a first Office Action on the merits. No certification or fee is required.

The references were cited by or submitted to the U.S. Patent and Trademark Office in parent application Serial No. 08/374,645,

Serial No. sn unknown

filed April 27, 1995, which is relied upon for an earlier filing date under 35 USC 120. Thus, copies of these references are not attached. 37 CFR 1.98(d).

Respectfully submitted,

LOWE, PRICE, LEBLANC, & BECKER

Robert L. Price Registration No. 22,685

99 Canal Center Plaza, Suite 300 Alexandria, Virginia 22314 (703) 684-1111 RLP:kmb Date: March 12, 1997 Facsimile: 703-684-1124

SHEET \_L OF \_L ATTY, DOCKET NO. 1451-007A T OF ART CITED BY APPLICANT APPLICANT Kenneth J. McNAUGHT et al FILING DATE GROUP (PTO-1449) March 12, 1997 U.S. PATENT DOCUMENTS FILING CLASS SUBCLASS DATE PATENT NO. DATE NAME INITIALS 106 213 8/28/92 4/5/94 Fergason et al 5,300,145 4m FOREIGN PATENT DOCUMENTS Translation EXAMINER'S CLASS SUBCLASS PATENT NO. COUNTRY Yes No DATE INITIALS 12/24/92 Australia PL 0537 9/12/84 EPC 0.118 240 OTHER ART (Including Author, Title, Date, Pertinent Pages, Etc.) Fractionation and Characterization of Dent Corn and Amylomaize Starch Granules by J.E. Cluskey, C.A. Knutson and G.E. Inglett, Peoria - Starch/Starke 32(1980) Nr. 4.S. 105-109 Ceral Chemistry; Vol. 52, No. 6, Nov-Dec, 1975 Chemical Characteristics and Physico-chemical Properties of the Extruded Mixtures of Cereal Starches by L.Fornal et al, Starch/Starke 39 1987) Nr. 3, p 75-78 Corn Harndess Determination, Y.Pomeranz et al; Cereal Chem 61(2):174-150, vol 61, No.2, 1984 DATE CONSIDERED EXAMINER.

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

# INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5:		(1)	) International Publication Number:	WO 94/03049
A01H-5/10, C08B 30/00 A23L 1/308, 1/0522	A1	(43	) International Publication Date:	17 February 1994 (17.02.94)
(21) International Application Number: PCT/AU (22) International Filing Date: 30 July 1993			(74) Agent: F.B. RICE & CO.; 28/ NSW 2041 (AU).	A Montague Street, Balmain,
(30) Priority data: PL 3894 PL 7266 31 July 1992 (31.07.92) PL 7266 12 February 1993 (12.02.		AU AU	(81) Designated States: AU, CA, J (AT, BE, CH, DE, DK, ES MC, NL, PT, SE).	P, NZ, US, European palent , FR, GB, GR, IE, IT, LU,
(71) Applicant (for all designated States except US): GO FIELDER INGREDIENTS LIMITED [AU/ vel.4, 230 Victoria Road, Gladesville, NSW 21	'AUI: I	Le-I	Published With international search rep	or.
(72) Inventors; and (75) Inventors/Applicants (for US only): McNAUGHT, J. [AU/AU]; 18 Marcella Street, North Eppi 2121 (AU). MALONEY, Eric [AU/AU]; 19 Street, Tamworth, NSW 2340 (AU). BROWN [AU/AU]; 2 Melissa Avenue, Tamworth, N: (AU). KNIGHT, Adrian, Timothy [AU/AU]; dah Street, Lane Cove, NSW 2066 (AU).	ng, NS Brisba I, Ian, SW 23	W III L		
(ED Tid IIICII 'A'AG CET ETI DEL AND CETE				

(54) Title: HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

#### (57) Abstract

Hybrid maize seeds which yield a starch having an amylose content of 80 % or more are disclosed as are starches having that amylose content. Compositions including these high amylose starches are also disclosed. Fractions of high amylose starches which have been formed on the basis of granule size are shown to have enhanced dietary fibre and/or resistant starch content. Such fractions enable the preparation of food compositions of enhanced dietary fibre and/or resistant starch content.



# UNITED STATES DEPARTMENT OF COMMERCE Patent and Trademark Office

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Weshington, DC 20231

ATTORNEY DOCKET NO. FIRST NAMED INVENTOR FILING DATE 03/12/97

HH21/1002

LOWE PRICE LEBLANC & BECKER 99 CANAL CENTER PLAZA SU1TE 300 ALEXANDRIA VA 22314

EXAMINER

PAPER NUMBER

5 10/02/96

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

\$U.S.GOVERNMENT PRINTING OFFICE 1993-319-826

1-File Com

Office Action Summary	Application No. 08/815,763	Applicant(:	i) McNaught	et al.
Office Action Summary	Examiner Thomas Ha	ns	Group Art Unit 1649	
☐ Responsive to communication(s) filed on				
☐ This action is FINAL.				
Since this application is in condition for allowance exc in accordance with the practice under Ex parte Quayle	ept for formal matters , 1935 C.D. 11; 453	, prosecuti D.G. 213.	on as to the mer	rits is closed
A shortened statutory period for response to this action is is longer, from the malling date of this communication. F application to become abandoned. (35 U.S.C. § 133). E 37 CFR 1.136(a).	ailure to respond withi	n the neric	d for reconned u	will cause the
Disposition of Claims				
☑ Cialm(s) <u>1-7</u>		is/are	pending In the a	pplication.
Of the above, claim(s)		ls/are w	vithdrawn from c	consideration.
Claim(s)				•
		i	s/are rejected.	
☐ Claim(s)				) <b>.</b>
☐ Claims				
Application Papers  See the attached Notice of Draftsperson's Patent Di The drawing(s) filed on	objected to by the Exa	miner.	Hisapproved.	
Priority under 35 U.S.C. § 119  Acknowledgement is made of a claim for foreign pri All Some* None of the CERTIFIED con received. received in Application No. (Series Code/Seria received in this national stage application from *Certified copies not received:	oles of the priority doci	uments hav	ve been . · lule 17.2(e)).	···································
□ Acknowledgement is made of a claim for domestic particle.  Attachment(s)     □ Notice of References Cited, PTO-892     □ Information Disclosure Statement(s), PTO-1449, Paper Interview, Summary, PTO-413     □ Notice of Draftsperson's Patent Drawing Review, PT	per No(s)4	C. § 119(e)		
SEE OFFICE ACTION	ON THE FOLLOWING P	AGES		

U. S. Patent and Trademark Office PTO-326 (Rev. 9-95)

Office Action Summary

Part of Paper No. 5

Application/Control Number: 08/815,763

Page 2

Art Unit: 1649

#### DETAILED ACTION

#### Claim Rejections - 35 USC § 112

I. Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The invention employs a novel corn lines. Since these plants are essential to the claimed invention it must be obtainable by a repeatable method set forth in the specification or otherwise be available to the public. If the plants are not so obtainable or available, the requirements of 35 USC 112 may be satisfied by the deposit of the plants. The specification does not disclose a repeatable process to obtain the plant and it is not apparent if the plants are readily available to the public. Thus, a deposit is required for enablement purposes. A deposit of 2500 seeds of each of the claimed embodiments is considered sufficient to ensure public availability. If the deposit is made under the terms of the Budapest Treaty, then an affidavit or declaration by applicants, or a statement by an attorney of record over his or her signature and registration number, stating that the specific strain has been deposited under the Budapest Treaty and that the strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent, would satisfy the deposit requirement made herein.

Application/Control Number: 08/815,763

Page 3

Art Unit: 1649

If the deposit has not been made, under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 C.F.R. 1.801-1.809, applicants may provide assurance of compliance by an affidavit or declaration, or by a statement by an attorney of record over his or her signature and registration number, showing that:

- (a) during pendency of this application, access to the invention will be afforded to the Commissioner upon request;
- (b) all restrictions upon availability to the public will be removed upon granting of the patent;
- (c) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request or for the effective life of the patent, whichever is longer;
- (d) a test of viability of the biological material at the time of the deposit (see 37 CFR 1.807); and,
- (e) the deposit will be replaced if it should ever become inviable.
- 2. Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for hybrid maize seed resulting from crosses of the recited maize lines (G112, G112, G116, etc.), does not reasonably provide enablement for any other hybrid maize seed. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

  Claim 1 reads broadly on hybrid maize seed produced by crossing any maize plants.

  The specification of the instant application teaches crosses between known maize lines comprising the ac amylose extender genes. It does not teach or suggest how one of skill in the art can cross

Case 1:04-cv-01443-GMS

Application/Control Number: 08/815,763

Page 4

Art Unit: 1649

maize plants that do not comprise the ae genes and produce hybrid maize plants with starch that is 80% amylose. Furthermore, it is known in the art that amylose production in corn seed is affected by factors other than the genetic makeup of the plant. Factors such as location of the kernel on the ear, growing location and drought conditions each may affect amylose content. The specification fails to address these factors, any of which add to the inherent unpredictability of producing grain with starch that is 80% amylose. Given the lack of guidance provided by the specification and the breadth of the claim, claim 1 is rejected under 35 U.S.C. 112, first paragraph.

#### Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was

Application/Control Number: 08/815,763

Page 5

Art Unit: 1649

made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over each of Vineyard and Bear (Corn Genetics Cooperation News Letter 26:5 (1952)), Friedman et al. (US Pat. No. 4,798,735) and Robertson et al. (US Pat. No. 5,00,864).

Claims 1-7 are directed to hybrid maize seeds comprising starch that is at least 80% amylose.

Vineyard and Bear teach that maize plants comprising the amylose extender gene can have a range of amylose from about 50% to 80%.

Friedman et al. teach a maize plant comprising the ae gene which has a high percentage of amylose (columns 1-4). Fried et al. also teach that starch with high amylose is desirable commercially.

Robertson et al. teach a dominant amylose extender mutant of maize (see entire document). Seeds from this mutant produce and high amylose starch.

It would have been obvious to one of ordinary skill in the art to use maize lines comprising amylose extender mutants in traditional breeding methods in order to produce seeds with high amylose starch, especially in light of the teachings of each of Vineyard and Bear, Friedman et al. and Robertson et al. One of ordinary skill in the art would recognize that hybrid maize lines that produce seeds with high amylose starch were available and could be used to produce hybrid lines which produce seeds with high amylose starch. The actual percentage of amylose would

Page 6

Application/Control Number: 08/815,763

Art Unit: 1649

necessarily be increased as one of ordinary skill in that art would select for plants which produced seed with high amylose starch. Thus, claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over each of Vineyard and Bear, Friedman et al. and Robertson et al.

#### Conclusion

#### 5. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thomas Haas whose telephone number is (703) 305-7270. The examiner can normally be reached on Mon.-Fri. from 7:00 to 4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Doug Robinson, can be reached on (703) 308-2897.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

> Douglas W. Robinson Supervisory Patent Examiner Technology Center 1600

U. S. Patent and Tredemark Office. PTO-892 (Rev. 9-95)

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Notice of References Cited

Exhibit B 0050

Part of Paper No. 5

Form PTO 948 (Rev. 10-94)

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8/8/5763

# NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

PTO Draftpersons review all originally filed drawings regardless of whether they are designated as formal or internal. Addition 5. patent Examiners will review the drawings for compliance with the regulations. Direct telephone inquiries concerning this review to the Drawing Review Branch: 703-305-8404.

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Docket No.: 1451-007A (50179-025)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Kenneth J. McNAUGHT et a Serial No. 08/815,763 Filed: March 12, 1997

APR 0 2 1999 5

Group Art Unit: 1649
Examiner: T. Haas

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

#### PETITION FOR EXTENSION OF TIME

Honorable Commissioner of Patents and Trademarks Washington, D. C. 20231

Sir:

For:

Applicants petition the Commissioner of Patents and Trademarks to extend the time for response to the Office Action dated October 2, 1998 for three months from January 2, 1999 to April 2, 1999.

Please charge the \$870.00 fee to cover the cost of the extension to Deposit Account No. 500417. Any deficiency or overpayment should be charged or credited to Deposit Account No. 500417.

Respectfully submitted,

MCDERMOTT, WILL & EMERY

Robert L. Price

Registration No. 22,685

04/05/1999 SARAYA 00000047 500417 08815763 01 FC:117 870.00 CH

> 600 13th Street, N.W. Washington, DC 20005-3096 (202) 756-8000 RLP:ajb Date: April 2, 1999 Facsimile: 202-756-8087

50179-025 (1451-007A) Docket No.:

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Kenneth J. McNAUGHT et

Serial No. 08/815,763

Filed: March 12, 1997

Group Art Unit:

Examiner: T. Haas

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

# AMENDMENT

Honorable Commissioner of Patents and Trademarks Washington, D. C. 20231

Sir:

-126

In response to the Official Action of October 2, 1998, please amend the application as follows:

# IN THE CLAIMS:

Please cancel claims 1-7 from the application and replace with the following new claims 8-20:

B. Hybrid maize seed carrying recessive ae amylose extender gene and containing starch having an amylose content of greater than 80% by weight.

The hybrid maize seed according to claim & containing starch having an amylose content of greater than 85% by weight.

1

Case 1:04-cv-01443-GMS

Serial No. 08/815,763

The hybrid maize seed according to claim & containing starch having an amylose content of 90.1% weight.

The hybrid maize seed according to claim 8/containing starch having an amylose content of 94.8% weight.

Hybrid maize seed according to claim produced by crossing inbred maize plants carrying the ae amylose extender gene.

The hybrid maize seed according to claim 8 produced from F1 maize plant hybrids.

14. The hybrid maize seed according to claim 8, which has all the characteristics of ATCC Accession No. 75182.

45. Hybrid maize seed carrying recessive ae amylose extender gene deposited under ATCC Accession No. 75182.

Majze plants grown from the hybrid maize seeds according to claim 25.

Viable maize seeds and plants and succeeding generations thereof grown from seeds deposited under ATCC Accession No. 75182, and maize seeds and plants carrying recessive ae amylose extender

gene transferred from the deposited seeds and capable of producing starch having an amylose content of greater than 80% by weight. 10

 $\mathcal{M}$ . Viable maize seeds and plants according to claim  $\mathcal{M}_{\mathcal{L}}$ capable of producing starch having an amylose content of greater than 85% by weight. 10

12 29: Viable maize seeds and plants according to claim 27 capable of producing starch having an amylose content of 90.1% by weight.

10 28. Viable maize seeds and plants according to claim 17 capable of producing starch having an amylose content of 94.8% by weight .--

#### REMARKS

The Official Action of October 2, 1998 has been carefully considered. Accordingly, the amendments presented herewith for the application; taken with the following remarks, are believed sufficient to place the application in condition for allowance.

By this amendment, claims 1-7 are cancelled from the application and replaced with new claims 8-20. These new claims are clearly based on the specification and presented to overcome the objections under 35 U.S.C. §112 set forth in the Official Action. Accordingly, entry of the claims is requested.

In the Action, original claims 1-7 were rejected under 35 U.S.C. §112 as referring to novel corn lines which, according to the Examiner, required a deposit of the plants. Accordingly, claims have been revised so that main claim 8 is now directed to hybrid maize seeds which carry the recessive as amylose extender gene and containing starch which has an amylose content of greater than 80% by weight. Independent claim 14 specifically refers to the deposited F1 hybrid which has been deposited with ATCC under designation No. 75182 as discussed on page 8 of the specification. Independent claim 16 also refers to this ATCC Accession No. 75182.

It is submitted that the claims as revised are in full compliance with 35 U.S.C. §112. Independent claim 8 covers only those hybrid maize seeds which carry the recessive ae amylose extender gene. This gene is referred to, for example, in the first

paragraph on page 2 of the specification and further discussed at page 8 of the specification. Accordingly, main claim 8 is fully enabled by the present specification.

In paragraph 2 of the Action, claim 1 is also rejected under 35 U.S.C. \$112, first paragraph because the specification, while being enabling for hybrid maize seed resulting from crosses of the recited maize lines, is not considered to provide enablement for any other hybrid maize seed. This rejection is also traversed and reconsideration is requested in view of the amended claims.

As the Examiner will note, the new claims do not now broadly read on hybrid maize seed produced by crossing any maize plants. The hybrid maize seed of claim 8 is readable only on those maize seeds which carry the recessive ae amylose extender gene and contains starch having an amylose content of greater than 80% by weight. Claims 14 and 15 refer specifically to the deposited seed of ATCC 75182 or seeds having the characteristics of that deposited seed. Claim 16 claims maize plants grown from the seed of claim 15 and claims 17-20 cover viable seeds and plants. Therefore, it is submitted that this rejection under 35 U.S.C. §112 has been overcome and reconsideration is requested.

With respect to both of the rejections made under 35 U.S.C \$112, the Examiner will note that the specification describes a number of F1 hybrids in the specification which enable the present invention. For example, at the top of page 9, there are set forth

experimental hybrids which yield starches obtained from crosses between the various parent lines which have high amylose contents. The deposit has been made in the ATCC 1992 under Accession No. 75182, of a hybrid which is representative of the hybrid maize seeds of this invention. However, the application is not so limited in view of the description and enablement of various other hybrid maize seeds. Further, the Examiner is advised that seeds of code 008 which have been deposited with ATCC in 1992 has been grown extensively commercially in Australia and is available commercially so that anyone can obtain samples of these seeds. This commercial availability causes the specification and claims to clearly be in conformance with 35 U.S.C. §112. Therefore, reconsideration of the rejection under 35 U.S.C. §112 is respectfully requested.

In the Action, the claims of the application also stand rejected as unpatentable over references identified as those of Vineyard and Bear (Corn Genetics Cooperation News Letter 26:5 (1952)), Friedman et al. U.S. Patent No. 4,798,735 and Robertson et al. U.S. Patent No. 5,004,864. It is the Examiner's position that these references disclose maize plants and it would be obvious to one of ordinary skill in the art to use the maize plants of the prior art with traditional breeding methods to produce seeds with amylose starch. These rejections are respectfully traversed and reconsideration is requested.

While the high amylose maize seed lines disclosed and claimed in this application were developed using methods of pedigree selection, with Applicants' skill and knowledge, it is submitted that the references relied on by the Examiner do not show the motivation required by the law to desire to produce seeds which meet the requirements of Applicants' claims. Ex parte Levengood, 27 USPQ 2d 1300. Where is the suggestion in the references to produce seeds of the type claimed? Applicant submits it cannot be found in the references.

This invention was made by Applicants from their laboratory data, wherein ears selected from "high" levels of amylose were earrowed in the nursery, selfed, selected for visual traits, re-tested in the laboratory and re-planted in subsequent nurseries. The seed lines were bulked when the rows were homozygous and test crosses were made to determine hybrid performance. The test-cross hybrids were evaluated for both yield and agronomic characteristics and the selfed pure seed samples were analyzed in the laboratory for amylose levels. This close cooperation between the field and the laboratory contributed to the genetic gain for amylose level. Backcrossing was also used to increase the amylose level of inbred seeds that possessed other superior traits, particularly agronomic and seed production traits.

The maize seed lines exemplified in the present disclosure and claimed herein all contained the ae amylose extender gene (as

specified in the claims) which were combined in a number of inbred lines, which gave amylose contents of greater than 80% amylose. The greater than 80% amylose hybrids were prepared to perform under defined agronomic conditions, which maximized yield. The hybrid G116 x G121 (code 008 and deposited at ATCC), which has been commercially grown since 1992, has been evaluated in a range of climatic conditions, tropical to temperate and the starch found to vary by no more than 2% in amylose content. These results have been obtained through careful crop management in a manner known and appreciated by persons skilled in the art who understand the cultivation of high amylose maizes. The specification for the commercial starch, Hi-maize<sup>™</sup>, which resulted from this invention has requirements for both amylose (greater than 80% on a dry solids basis) and total dietary fibre levels which have been met since commercial usage was commenced in 1993.

It is submitted, however, that the references relied on by the Examiner do not teach or suggest these techniques and more importantly, do not contain any suggestions of the desirability of producing the seeds and plants of the present invention, <u>In real Antonie</u>, 195 USPQ 6; <u>In re Kratz</u>, 201 USPQ 71. Obvious to try is not a standard of patentability, <u>In re Yates</u>, 211 USPQ 1149.

The prior art relied on by the Examiner does not provide any disclosure of hybrid seeds having the genetic make-up or physical characteristics of the seeds according to the present invention.

The Examiner suggests that Vineyard and Bear 1952, disclose starch with up to 80% amylose, Friedman et al. U.S. Patent 4,798,735 disclose only 67.5% amylose and Robertson et al. U.S. Patent 5,004,864 disclose 72% amylose. However, the prior art does not disclose or teach how to obtain maize seeds according to the present invention which have an amylose content of greater than 80% up to 95.7% as disclosed in the examples provided by the present inventors. Furthermore, the Examiner's attention is directed to the fact that the example of Friedman et al. is not for seeds having the recessive "ae" amylose extender gene alone but in combination with the sugary-2 " $su_2$ " gene which provides a starch which is clearly different in structure and performance to that produced by the amylose extender ae gene on its own. Similarly, the dominant amylose extender "AE" gene has a different mode of biochemical action to that described by the recessive ae gene as would be appreciated by a person knowledgeable in this area.

The Examiner states that it is predictable that a person could continue to increase the amount of amylose present in the maize to greater than 80% based on the teachings of Vineyard and Bear (1952), but this has not been the case for any of the documents cited by the Examiner. In fact, the law requires that the references themselves must disclose this predictability, In re Reinhart, 189 USPQ 143. A reference which provides only general guidance is not sufficient, Ex parte Obukowicz, 27 USPQ 2d 1063

(1993). Therefore, this indicates that people of ordinary skill in the art could not achieve the result documented and claimed in this patent application.

As the Examiner will appreciate from the above discussion of the development of applicant's invention and the limitations of the references, it is clear that none of the references describe or suggest hybrid maize seeds which carry the recessive ae amylose extender gene and contain starch having an amylose content of greater than 80% by weight. In fact, other claims, such as claims 9, 10 and 11 cover hybrid maize seeds which have amylose contents of greater than 85%, 90.1% and 94.8%, respectively. Applicants submit that there is clearly no teaching or motivation in the art to produce hybrid maize seeds and plants of the type claimed herein. There is clearly nothing in any of the references themselves to suggest that hybrid maize seeds which carry the amylose extender gene and contains starch having an amylose content of greater than 80% by weight was ever recognized by the prior art or enabled by the prior art. For these reasons, it is submitted that the claims are clearly patentable thereover.

It is believed that the above represents a complete response to the Official Action and serves to place the application in condition for allowance.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage

in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

McDERMOTT, WILL & EMERY

Robert L. Price Registration No. 22,685

600 13th Street, N.W. Washington, DC 20005-3096 (202) 756-8000 RLP:brca:ajb Date: March , 1999 Facsimile: 202-756-8087



UNITED STAT DEPARTMENT OF COMMERCE Patent and Trademark Office

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

カクロシブブ

MCDERMOTT WILL & EMPLEY 600 13TH STREET NW WASHINGTON DC 20005-3094

ſ	APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART U	NIT	DATE MAILED
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THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.

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- III. All communications regarding this application must give application number and batch number. Please direct all communications prior to Issuance to Box ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

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1. S. Patent and Trademark Office PTO-37 (Rev. 9-95)

X Examiner's Statement of Reasons for Allowance

Notice of Allowability

Part of Paper No. \_\_\_\_8\_

Application/Control Number: 08/815,763

Page 2

Art Unit: 1649

In accordance with rule 126, claims 8-20 have been re-numbered 24-36 for the purpose of examiniation.

Reasons for allowance.

The following is an examiner's statement of reasons for allowance:

The prior art fails to teach or reasonably suggest hybrid maize plants comprising recessive amylose extender genes and having a starch with an amylose content of 80%.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thomas Haas whose telephone number is (703) 305-7270. The examiner can normally be reached on Mon.-Fri. from 7:00 to 4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

LYNETTE R, F. SMITH SUPERVISORY PATENT EXAMINER TECHNOLOGY CENTER 1600

 $f_{P_{n}}(x, \overline{x}) \tilde{f}$ 93 PATENT Docket No.: 50179-025 IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

BATCH NO.: U95

KENNETH MCNAUGHT, et al.

Serial No.: 08/815,763

Group Art Unit: 1649 Allowed: June 23, 1999

Filed: March 12, 1997

Examiner: T. Haas

For: HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

## LETTER SUBMITTING FORMAL DRAWINGS

Box Issue Fee Assistant Commissioner for Patents Washington, DC 20231

Sir:

In response to the Notice of Allowability dated June 23, 1999, submitted herewith are four (4) sheets of Formal Drawings in connection with the above referenced application.

Respectfully submitted,

Corst ng

Robert L. Price Registration No. 22,685

600 13th Street, N.W. Washington, DC 20005-3096 (202) 756-8000 RLP:th Date: July 13, 1999 Facsimile: (202) 756-8087

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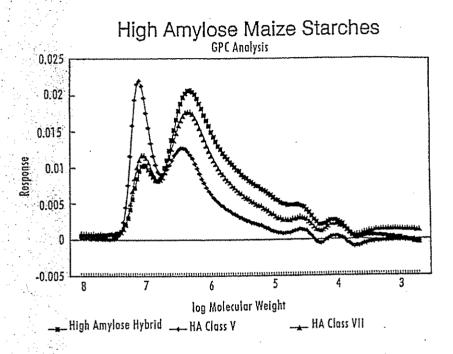


FIG. 1

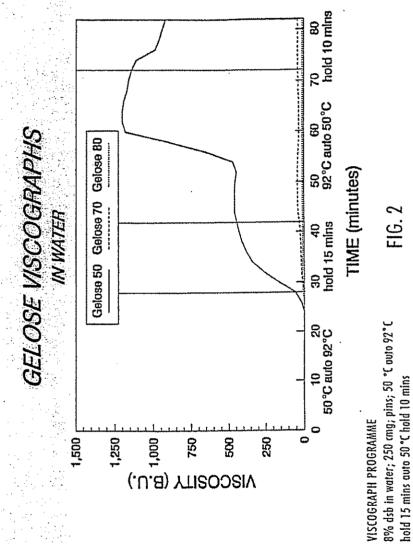
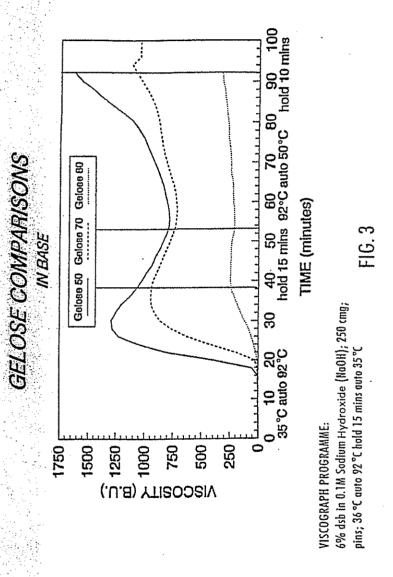


Exhibit B 0069



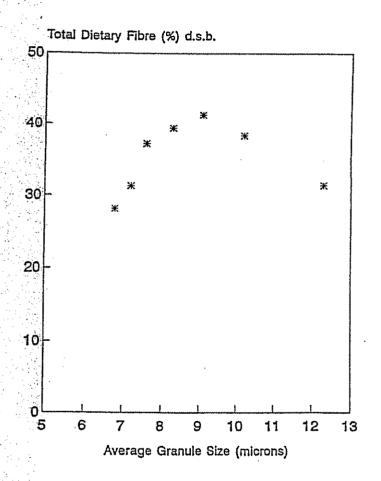


FIG. 4

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